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STREPTOTHRIX IN BRONCHOPNEUMONIA OF RATS SIMILAR TO THAT IN RAT-BITE FEVER*

PLATES 18 TO 20

RUTH TUNNICLIFF

From the Memorial Institute for Infectious Diseases, Chicago

Hektoen[1] has described the various lesions of bronchopneumonia in white rats, a disease which appears to be of frequent occurrence. The same lesions have also been observed in the gray rat. In the course of a study of this condition, especially as it occurs in the white rat, a streptothrix was found which may be of interest in connection with rat-bite fever in man.

Sixty white rats showing acute or chronic bronchopneumonia were examined. A long, fine, straight or wavy filamentous organism was observed in smear preparations or by dark-field illumination in 56. No branching was seen in these specimens. Three lesions in which the organism was not found, were chronic. It was most abundant in acute lesions. It is a gram-negative organism, nonacidfast, and it stains fairly distinctly with carbol-gentian-violet and Giemsa stain. It is distinguished from cilia by its greater length, more pointed ends, and its occasional wavy form. In the dark field I observed once or twice a slow worm-like motion of the filamentous organism, and once a cork-screw motion. The organism is barely visible in the tissues stained with methylene blue, but is distinct in tissues impregnated with silver nitrate according to Levaditi's method. Here, it appears more wavy and more fragmented than in any other preparations. It is seen inside the bronchi and sometimes in the surrounding tissue when the infection extends there. This organism was not observed in 24 normal rats. Short curved gram-negative organisms were seen in smears from the lesions of 3 rats; diplococci in 4 and diphtheroid bacilli in 1.

The rats after being killed with chloroform were generally washed with Harrington's solution and the lungs removed with sterile instruments. If the tissues were not taken out aseptically, the surfaces were seared, and the mucoid or purulent contents of the bronchi withdrawn by a sterile pipet. Pieces of the lung containing lesions were also used for inoculation. Anaerobic cultures (Wright's pyrogallic-acid and sodium-hyroxid method or liquid petrolatum) were made in ascitic agar containing tissue, ascitic fluid and tissue, and semicoagulated horse serum with and without tissue—the media commonly used

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* Received for publication August 16, 1916.

[1] Tr. Chicago Path. Soc., 1916, 10.

for growing spirochetes—but no spirochetes were cultivated. Aerobic and anaerobic cultures were also made on blood agar and the ordinary laboratory media.

A streptothrix resembling the organisms seen in the smear preparations from the lung was isolated from 20 rats, in pure growth 13 times. Cultures from 33 rats remained sterile. Why successful cultures were obtained from some rats and not from others is undetermined. The character of the lesion does not seem to make any difference. Successful cultures were more frequent in the case of sick rats and of lesions in which other bacteria were present.

The other organisms isolated from 22 rats were Streptococcus viridans (6), and pyogenes (1), pneumococcus (2), staphylococcus (1), diphtheroid bacilli (3), anaerobic spirilla (3), an unidentified profusely growing gram-negative bacillus (4), and the colon bacillus (2). The last two were probably contaminations, as they were isolated also from some of the normal rats. Seven of these were isolated from cultures in which the streptothrix did not grow. Successful cultures of the streptothrix were grown on human-blood agar or ascitic goat-blood agar.

CULTURAL CHARACTERISTICS

This streptothrix is a facultative anaerobe, growing best at 37 C. It seems to grow as well with air as without. Slight growth appears at room temperature after several days' incubation. Growth generally appears in from 48 to 72 hours, in subculture in 24, as discrete, colorless or grayish, pinpoint, circular, elevated colonies, with sharp margins. At first they appear dull, but later they become glistening. They may also increase slightly in size. The medium is not changed. The growth in the fluid of condensation and in ascitic broth is white and flocculent, the medium remaining clear. The growth on Loeffler's blood serum is similar to the growth on blood agar, but does not always occur, probably because of difference between the lots of media. Three strains were grown on various litmus sugar media, to which ascitic fluid had been added (1:4). Growth occurs in inulin, lactose, maltose, mannite, raffinose, saccharose, and salicin. A slightly acid reaction is produced by all in maltose and by 1 in salicin. There is no growth on potato, plain and dextrose agar, ascitic dextrose agar, in plain and dextrose broth, or in milk.

Cultures are viable at 37 C. for about 14 days after growth is established. When the organisms are first isolated they often die after a few days' incubation.

No streptothrix has been grown from the lungs of the normal rats examined.

MORPHOLOGY AND STAINING PROPERTIES

In cultures the organism is slender, straight or wavy, filamentous, varying greatly in length, and showing fragmentation and bacillus- and coccus-like forms. Some branching is seen in young cultures, but not with especial frequency. Spindle- or irregularly shaped swellings and involution forms are often observed. Twisted and wavy forms are more frequent on solid media than in ascitic broth, where the organisms appear generally as long threads or chains or as adherent masses of bacilli (Fig. 6). They do not show a radial arrangement at the periphery of the colony. They stain with ordinary stains. They are gram-negative, except the swellings, and are not acidfast. The organism here is nonmotile.

ANTIBODY-PRODUCTION

An increase in opsonins and agglutinins for this organism has been observed in infected rats. For example, in one experiment the normal rat had a phagocytic index of 4, the two infected rats indexes of 12 and 20 respectively. The same control showed macroscopic agglutinations at 1:20, while the sera of the infected rats agglutinated at 1:60. The agglutination mixtures were incubated 1 hour and placed in the ice box over night.

PATHOGENICITY

Five young rats were each inoculated intraperitoneally with 2 c.c. of a mixture of a 24-hour growth in ascitic broth and that on 2 blood-agar slants. The streptothrix had been isolated from the lung of a rat dying spontaneously. On the following day 1 rat died, showing acute lesions in the lungs. The streptothrix was observed in smears from the heart blood and the lung, and was isolated from the blood and the lung. On the 2nd day after inoculation, another rat, which was sick, was killed. It showed pneumonia on both sides, the lesions being more advanced than those in the first rat. Smears from the lungs showed a few filamentous organisms. Cultures from the lung and the blood were negative. A third rat, killed on the 6th day, showed small acute lesions. The streptothrix was observed in smears from the lung. It grew slightly anaerobically on goat-blood agar. A fourth rat was killed on the 8th day. Very small lesions were present in the lungs and a few organisms were observed in smears. They grew but slightly. The fifth rat, killed on the 9th day after inoculation, showed no lesions.

Twelve young rats not inoculated were all normal.

Later, 2 rats were each inoculated intraperitoneally with the growth from 20 c.c. of an ascitic-broth culture of the same strain. One killed the following day showed acute congestion and hemorrhages of the lung and pleura. Smears from the lung showed rather short streptothrices. Smears from the blood were negative. The cultures from the lung showed Streptococcus viridans. The second rat was inoculated again 7 days later with the growth from 40 c.c. of an ascitic-broth culture. It was sick the following day and was killed, but no lesions were found.

Two guinea-pigs were each inoculated intraperitoneally with 5 c.c. of a 24-hour ascitic-broth culture of the streptothrix isolated from the lung of inoculated Rat 1. Both guinea-pigs were ill the following day. One was killed, but no lesions in the lungs were found. There were small hemorrhages in the adrenals, which appeared swollen. The blood cultures were negative. The other guinea-pig was killed the next day. There was a collapsed spot in one lobe of the lung. No organisms were found. The cultures from the lung and blood were negative. Another guinea-pig inoculated intraperitoneally with the growth from 15 c.c. of an ascitic-broth culture remained well.

Two rabbits inoculated intravenously with the growth of the same organism on 2 blood-agar slants, died at the end of 3 and 4 weeks respectively. The lungs were normal.

A monkey (Macacus rhesus) was inoculated intravenously with the 24-hour growth from 200 c.c. of an ascitic-broth culture of another streptothrix, isolated from the lung of a rat dying spontaneously. The monkey's temperature rose the following day from 102.6 to 104.6+. The number of leukocytes rose from 8600 to 18,000. The monkey had a chill and diarrhea, and was very ill for 2 days. No eruption was observed. There was another rise in temperature with a slight increase in the number of leukocytes, affecting principally the polymorphonuclears, at the end of 4 weeks, and a similar rise in temperature and number of leukocytes at the end of 8 weeks. No eruption was seen and the monkey appeared perfectly well.

The anaerobic spirilla isolated from 3 rats were not pathogenic for white rats.

From these experiments the conclusion may be drawn that this streptothrix is slightly pathogenic for white rats, often producing acute lesions in the lungs, but is not pathogenic for rabbits or guinea-pigs. The organism has some pathogenicity for the monkey.

DISCUSSION

This streptothrix corresponds in general both morphologically and culturally with Streptothrix muris-ratti, isolated in pure culture from the blood of patients with rat-bite fever by Schottmüller[2] and Blake,[3] and seen in the blood during paroxysms by Tileston.[4] The descriptions of the organisms by the first two observers differ slightly. Schottmüller describes the color of the original colonies as grayish-black, without sharp edges, those in subculture on blood agar as grayish-white, and those on Loeffler's serum plates as colorless. Blake describes the color of the colonies on Loeffler's blood serum as whitish, those on ascitic agar as grayish-white. His colonies are sharply marginated. Schottmüller's organisms are "tinged bluish" with the Gram stain; Blake's are gram-negative. Blake gives a much fuller description of his organisms than Schottmüller. However, their organisms correspond so closely that it seems probable that they are identical.

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[2] Dermat. Wchnschr., 1914, 58, Suppl., p. 77.

[3] Jour. Exper. Med., 1916, 23, p. 39.

[4] Jour. Am. Med. Assn., 1916, 66, p. 995.

Blake, who kindly examined 3 strains of the rat streptothrix, found that it differs from his organism in growing a little less abundantly on Loeffler's blood serum and in showing more beaded and coccus-like forms. He found the appearance of the colonies on solid media, the growth in ascitic broth, and the other characteristics identical. He was unable to demonstrate any agglutination of the strains by the serum of his rat-bite-fever patient. One strain of the rat streptothrix differs from his in producing slight acidity in salicin. Blake did not use maltose ascitic-fluid broth, which was slightly acidified by the three strains of the rat streptothrix tested.

Futaki[5] and his associates describe a motile spirochete in the skin and lymph node of 2 cases of rat-bite fever, but details of their experiments and cultures are not available. Some of the spiral forms in my Levaditi specimens closely resemble the spirochetes shown in their drawing.

According to Crohn[6] there is no record at hand of examination of the rat inflicting the injury. He states that white rats and mice are not known to cause rat-bite fever. I therefore examined 28 wild rats and found 1 infected. The lesions in the lung were the same as those observed in the white rats, and showed the same organism. As the trachea of infected rats is full of mucus containing large numbers of the organisms, it is readily seen that a bite from such a rat might be infectious.

CONCLUSIONS

A streptothrix similar to Streptothrix muris-ratti, isolated from the blood of patients with rat-bite fever, has been observed in smears and isolated in pure culture from the lungs of rats with bronchopneumonia.

An increase in opsonins and agglutinins for this organism has been found in infected rats.

Acute lesions have been produced in the lungs of rats inoculated intraperitoneally with cultures of this organism.

[5] Jour. Exper. Med., 1916, 23, p. 249.

[6] Arch. Int. Med., 1915, 15, p. 1014.

EXPLANATION OF PLATES

PLATE 18

FIG. 1.—Smear from lung. Carbol-gentian-violet. × 1200.

FIG. 2.—Section through lung of rat with bronchopneumonia, showing the streptothrix. Levaditi preparation. × 1200.

FIG. 3.—A 24-hour growth of the streptothrix on ascitic-fluid goat-blood agar. Carbol-gentian-violet. × 1200.

PLATE 19

FIG. 4.—Section through bronchus showing mucus filled with streptothrices. Levaditi preparation. × 350.

FIG. 5.—Same as Fig. 4. × 1200

PLATE 20

FIG. 6.—A 24-hour growth in ascitic broth. Carbol-gentian-violet. × 1200.

FIG. 7.—A 24-hour growth on human-blood agar. Carbol-gentian-violet. × 1200.

Plate 18

Figure 1

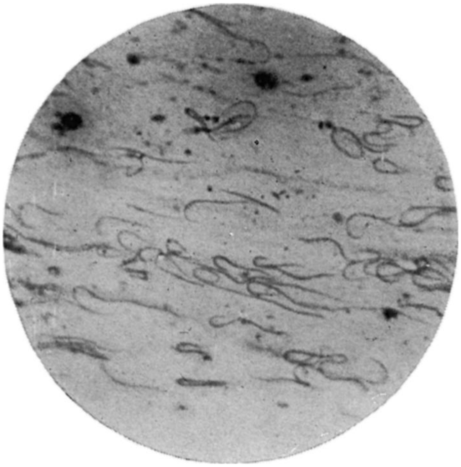
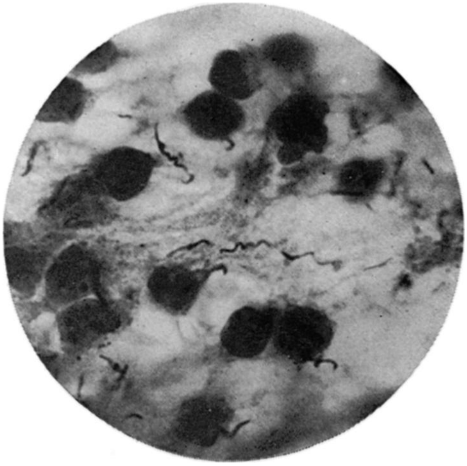


Figure 2



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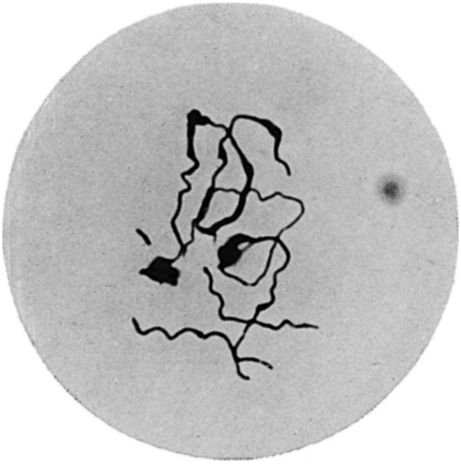
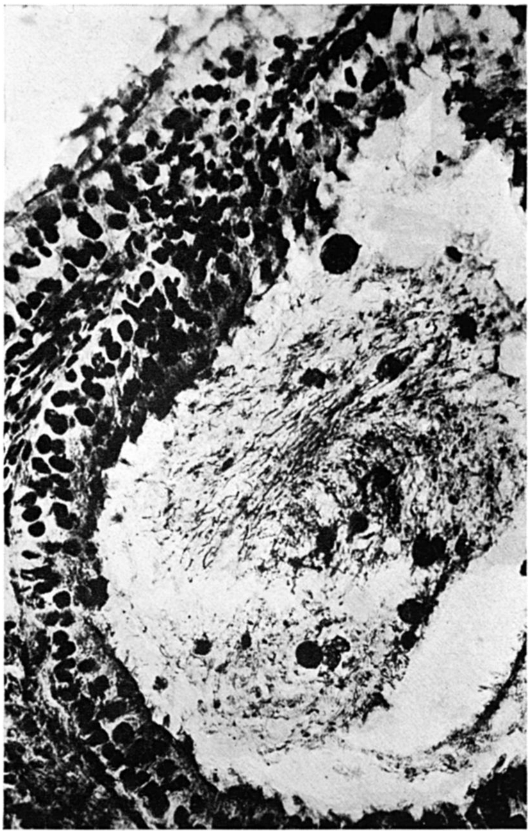


PLATE 19

Figure 4



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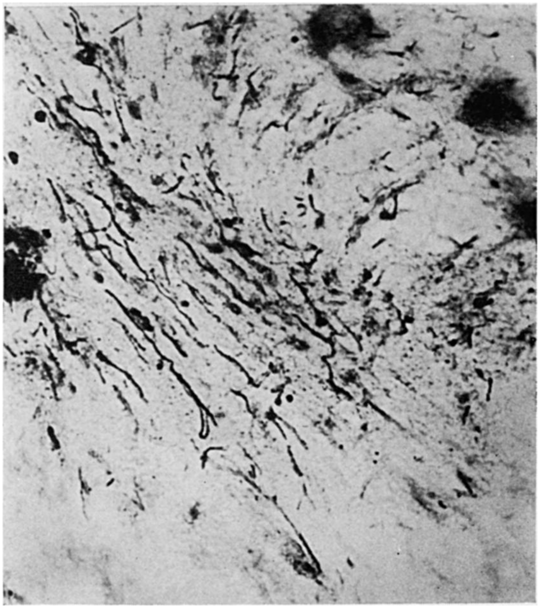

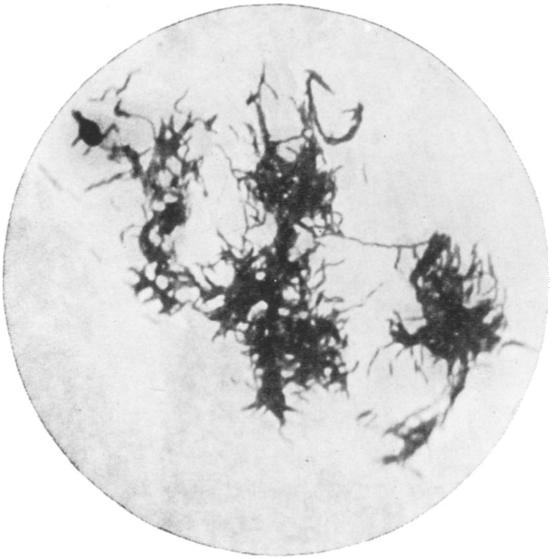

Figure 6

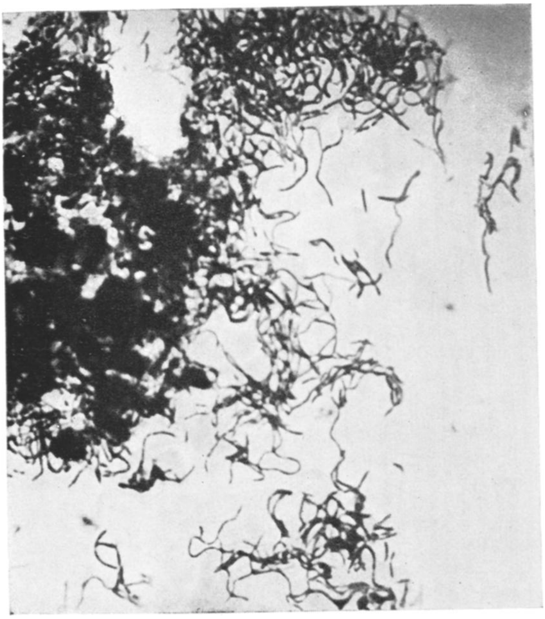

Figure 7